LETTER

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Seed coating with a neonicotinoid insecticide negatively affects wild bees

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Understanding the effects of neonicotinoid insecticides on bees is vital because of reported declines in bee diversity and distribution[1–3] and the crucial role bees have as pollinators in ecosystems and agriculture[4]. Neonicotinoids are suspected to pose an unacceptable risk to bees, partly because of their systemic uptake in plants[5], and the European Union has therefore introduced a moratorium on three neonicotinoids as seed coatings in flowering crops that attract bees[6]. The moratorium has been criticized for being based on weak evidence[7], particularly because effects have mostly been measured on bees that have been artificially fed neonicotinoids[8–11]. Thus, the key question is how neonicotinoids influence bees, and wild bees in particular, in real-world agricultural landscapes[11–13]. Here we show that a commonly used insecticide seed coating in a flowering crop can have serious consequences for wild bees. In a study with replicated and matched landscapes, we found that seed coating with Elado, an insecticide containing a combination of the neonicotinoid clothianidin and the non-systemic pyrethroid β-cyfluthrin, applied to oilseed rape seeds, reduced wild bee density, solitary bee nesting, and bumblebee colony growth and reproduction under field conditions. Hence, such insecticidal use can pose a substantial risk to wild bees in agricultural landscapes, and the contribution of pesticides to the global decline of wild bees[1–3] may have been underestimated. The lack of a significant response in honeybee colonies suggests that reported pesticide effects on honeybees cannot always be extrapolated to wild bees.

Neuroactive neonicotinoids are commonly used in seed coatings to control herbivorous insect pests in a variety of crops such as corn, cereals and oilseed rape and are taken up systemically by the growing plant and distributed to all tissues[5]. These chemicals account for more than one fifth of the world's insecticide market[14], and this widespread use requires that their effects on non-target organisms are investigated. A particular concern is the effect of neonicotinoids on bees[6,12], because of the bee's role in pollinating crops[4] and declines in bee diversity and distribution[1–3].

These concerns, together with research indicating negative effects of neonicotinoids on bees, have led to a European Union-wide restriction from 1 December 2013 on the use of the three neonicotinoids, clothianidin, imidacloprid and thiamethoxam, as seed coating in crops attractive to bees[6], to allow for studies on their environmental effects. Previous studies have mainly focused on the effects of neonicotinoids on bees artificially exposed to neonicotinoids[8–11], mostly on honeybees[11]. The key question is how wild bees, which may differ from honeybees in response to insecticides[15–17], are affected by neonicotinoids when foraging in real agricultural landscapes[11–13].

Here we investigated how seed coating oilseed rape with Elado (Bayer), including the systemic neonicotinoid clothianidin[5] and the non-systemic pyrethroid β-cyfluthrin[18] as active ingredients, influenced wild and managed bee species in Swedish agricultural landscapes. Because we assessed effects on bees under field conditions, our findings have important implications for policies regulating the use of neonicotinoids as well as for risk assessments of pesticides.

We designed a study with eight pairs of landscapes surrounding 16 geographically separated (>4 km) spring-sown oilseed rape fields (Fig. 1 and Extended Data Table 1). One field in each pair was randomly assigned to be sown with seeds coated with the dose of Elado recommended by the manufacturer and a fungicide, while the other field in each pair, the control field, was sown with seeds coated only with the fungicide. At these 16 fields we estimated: (1) the density of

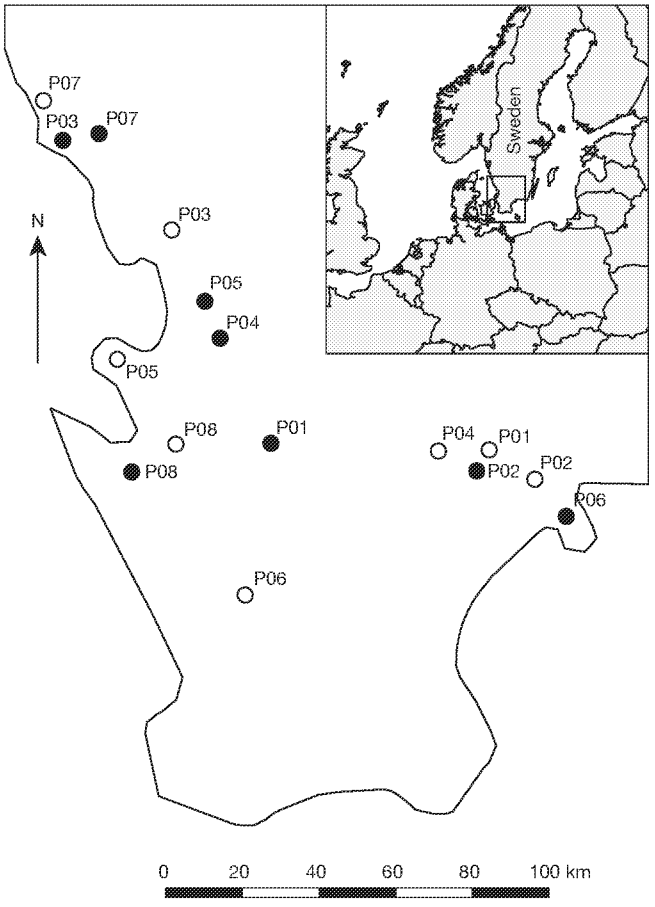

Figure 1 | Paired design with replicated landscapes. Location of the study area in southern Sweden and the eight pairs of landscapes (P01–P08) centred on oilseed rape fields sown with insecticide-coated (open circles) or untreated (control fields, filled circles) seeds. Pairing was based on land use within a 2-km radius surrounding the oilseed rape fields and geographical proximity between fields.

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wild bees; (2) the nesting activity of the solitary bee *Osmia bicornis* L.; (3) the colony development of the bumblebee *Bombus terrestris* L.; and (4) the colony strength of the European honeybee *Apis mellifera* L.

Our first finding was that the insecticide seed coating reduced the density of wild bees, that is, bumblebees and solitary bees, in the flowering oilseed rape fields and adjacent uncultivated field borders (generalized linear mixed model (GLMM), $F_{1,7} = 9.68$, $P = 0.019$; Fig. 2a and Extended Data Table 4). Wild bee density also increased with the size of the focal oilseed rape field, most probably because larger fields attract more bees or support larger colonies[19], but was not significantly related to the proportion of agricultural land in the surrounding landscape (Extended Data Table 4). Flower cover (number and size of flowers) of the oilseed rape had a positive influence on wild bee density ($F_{1,24} = 18.57$, $P < 0.001$) and was higher in fields sown with insecticide-coated seeds (Extended Data Table 5). However, the negative impact of the seed coating on wild bee density persisted irrespective of whether ($F_{1,7} = 9.68$, $P = 0.019$; Extended Data Table 4) or not ($F_{1,6} = 6.36$, $P = 0.044$) flower cover was included as a covariate in the statistical model.

Our second finding was that the insecticide seed coating correlated with reduced nesting of the solitary bee *O. bicornis*. To investigate this we placed three trap nests containing 27 *O. bicornis* cocoons (Extended Data Fig. 1) adjacent to each of the 16 fields before the beginning of oilseed rape flowering and monitored if emerging females started to build brood cells. In six of the eight control fields, but in none of the fields treated with the insecticide seed coating, females started to build brood cells (Wilcoxon test $Z = 2.84$, $P = 0.0045$; Fig. 2b). Although the reasons why the bees failed to build brood cells when exposed to the insecticide treatment remain unclear, a reduced capacity to navigate[8,9,20,21] is a possible explanation.

Our third finding was that the insecticide seed coating was negatively related to colony growth and reproduction of the bumblebee *B. terrestris*. Bumblebees are social and form colonies of one queen and tens or hundreds of workers. At each of the 16 oilseed rape fields we placed six commercially reared *B. terrestris* colonies (Extended Data Fig. 1). During their development, the bumblebee colonies are expected to grow in weight and worker force, and thereafter to switch to producing new queens and males with a resulting decline in colony weight[10]. The seed-coating treatment influenced the weight change of *B. terrestris* colonies (linear mixed model (LMM), day × day × treatment $F_{1,19} = 130.62$, $P < 0.001$, day × treatment $F_{1,21} = 143.00$, $P < 0.001$; Extended Data Table 6 and Fig. 3). As expected, *B. terrestris* colonies at control fields had an initial growth and a following decline (day × day $F_{1,28} = 114.70$, $P < 0.001$, day $F_{1,31} = 129.10$, $P < 0.001$), while those at fields with insecticide seed coating had a considerably smaller weight change ($F_{1,14} = 10.78$, $P = 0.0055$, $F_{1,16} = 0.92$, $P = 0.35$) (Extended Data Table 6 and Fig. 3). While the initial colony weight was the same in the two treatments (Extended Data Table 5), the rate of weight gain of colonies at fields with insecticide-coated seeds was lower than that of colonies at control fields ($F_{1,7} = 115.80$, $P < 0.001$; Extended Data Table 5). Effects of the treatment on colony development may result both from reduced pollen foraging efficiency and insufficient care for the brood[8,20–22]. Bumblebees have an annual life cycle where only the new queens produced at the end of the season hibernate and form new colonies the following spring. At the end of our experiment, fewer queen (GLMM, $F_{1,7} = 7.78$, $P = 0.027$) and worker/male cocoons (LMM, $F_{1,7} = 8.09$, $P = 0.025$) were produced at treated fields compared to control fields (Fig. 2c and Extended Data Table 5). These findings are in line with the reduced colony growth and 85% reduction in queen production reported for bumblebee colonies artificially exposed to imidacloprid under otherwise realistic conditions[8,10].

Our fourth finding was that the insecticide seed treatment had no significant influence on honeybee colony strength. In contrast to the *B. terrestris* colonies, the *A. mellifera* colonies did not differ in strength (number of adult bees) between the treatments after placement at the oilseed rape fields (LMM, $F_{1,7} = 0.01$, $P = 0.94$; Fig. 2d). This finding is

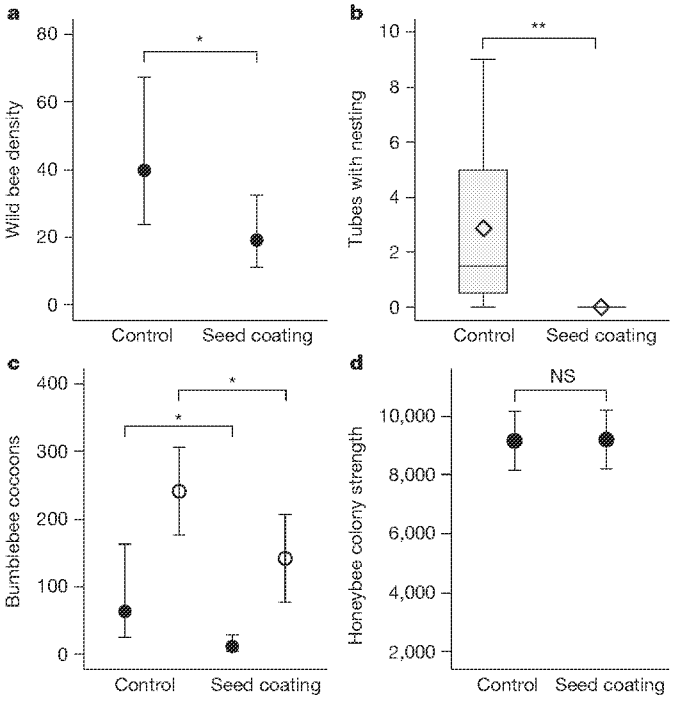

Figure 2 | Bee density and reproduction. **a–d**, Mean (± 95% confidence limits) number of wild bees (solitary bees and bumblebees) per 467 m^2 oilseed rape field and adjacent border (generalized linear mixed model (GLMM)) (**a**), median number of tubes per field with *O. bicornis* brood cells (Wilcoxon test) (**b**), mean (± 95% confidence limits) number of *B. terrestris* queen (filled circles, GLMM) and worker/male (open circles, linear mixed model (LMM)) cocoons per colony (**c**), and mean (± 95% confidence limits) number of adult *A. mellifera* per colony (colony strength) after placement at the fields (LMM) (**d**) in relation to treatment (control or insecticide seed coating) in the oilseed rape fields. $n = 8$ fields per treatment. Means and confidence limits are based on back-transformed, model-estimated least square means. In panel **b**, horizontal line in the box, open diamond symbols, boxes and whiskers indicate median, mean, 25th–75th percentiles and minimum–maximum, respectively. NS, not significant ($P > 0.05$); *$P < 0.05$, **$P < 0.01$.

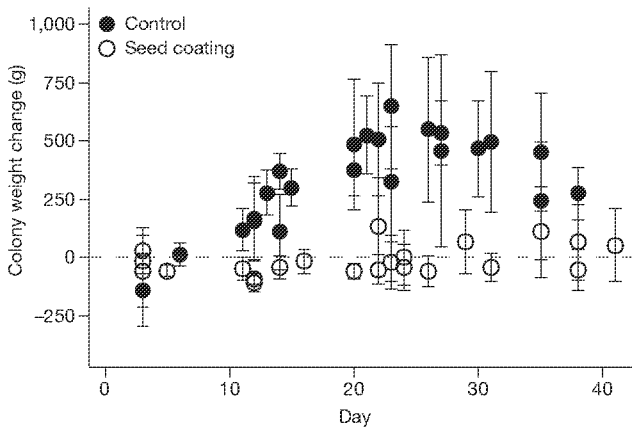

Figure 3 | Bumblebee colony development. Mean (± 95% confidence limits) bumblebee colony weight change (g) per field and survey day since day of placement at the fields (dashed horizontal reference line indicates initial colony weight) in relation to treatment (control (filled circles) or insecticide seed coating (open circles)). $n = 8$ fields per treatment. Dots are means of the six colonies at each field and weighing occasion. Two colonies at different fields (one control field and one treated field) were not weighed at one occasion, resulting in five colonies at those fields and weighing occasions. See Extended Data Table 6 for results from the colony growth model (linear mixed model).

Table 1 | Clothianidin concentrations in bee-collected pollen (ng g^{-1}) and nectar (ng ml^{-1}), and field border plants (ng g^{-1}), and tests of differences between treatments (control or insecticide-coated seeds)

	Control		Insecticide seed coating		Wilcoxon test for difference between treatments (n = 8*)	
	Range	Mean ± s.e.m.	Range	Mean ± s.e.m.	Z	P
Honeybee pollen	0	0	6.6–23	13.9 ± 1.8	−3.16	0.0016
Honeybee nectar	0–0.61	0.1 ± 0.1	6.7–16	10.3 ± 1.3	−3.40	<0.001
Bumblebee nectar	0	0	1.4–14	5.4 ± 1.4	−3.53	<0.001
Field border plants (≤2 days after sowing)	0	0	0–5.9	1.2 ± 0.8	−2.90	0.0037
Field border plants (2 weeks after sowing)	No material collected		0–6.5	1.0 ± 0.8		

*n = 6 for pollen collected by honeybees at control fields, because no such bees with pollen could be found at two fields; and n = 7 for field border plants collected within 2 days of sowing in both treatments, because of lack of communication regarding the sowing date between the farmer and the investigator collecting the samples.

in line with another field study[23] and previous work suggesting that honeybees are better at detoxifying after neonicotinoid exposure compared to bumblebees[17]. However, the lack of short-term effects does not preclude the existence of long-term effects of neonicotinoids[13].

Mass-flowering crops are valuable food resources for wild bees[19,24], but may act as ecological traps if foraging bees are exposed to pesticides such as neonicotinoids. To estimate exposure we assessed the transfer of clothianidin from plant to bee by first estimating the proportion of oilseed rape pollen collected by all three bee species, *O. bicornis*, *B. terrestris* and *A. mellifera* (Extended Data Table 6) and then quantifying the concentrations of clothianidin in bee-collected pollen and nectar (Table 1).

For *O. bicornis*, we found oilseed rape pollen in nine of 17 examined cells, accounting for 35.1 ± 17.0% (mean ± s.e.m.) of the collected pollen (Extended Data Table 5). Because there was no nesting activity at fields with insecticide-treated seeds, we could not assess pollen collection there. For *B. terrestris*, we found that in the 47 pollen samples collected from bees foraging in the oilseed rape fields, 80.1 ± 5.0% of the pollen was from oilseed rape, with similar results for both treated and control fields (Extended Data Table 5). For *A. mellifera* the pollen extracted from pollen traps mounted on the hives contained on average 57.8 ± 5.0% oilseed-rape-type pollen, with similar proportions for both treated and control fields (Extended Data Table 5).

We expected the insecticide seed coating to influence the amount of clothianidin that the bees were exposed to, but not β-cyfluthrin, since β-cyfluthrin, in contrast to clothianidin, is not systemically taken up by plants[5,18]. As expected, no β-cyfluthrin was detected (Extended Data Table 8), but both pollen and nectar collected by *A. mellifera* and nectar collected by *B. terrestris* foraging in the oilseed rape fields contained concentrations of clothianidin that were substantially higher in the treated fields than in control fields (Table 1). Clothianidin levels at treated fields were within the range of neonicotinoid levels quantified in pollen collected by honeybees in other studies (range: <0.1–912 ng g^{-1}; range of mean values per study and compound: <0.1–53.3 ng g^{-1})[25]. We also found higher clothianidin concentrations in plants collected in field borders adjacent to treated fields than adjacent to control fields, a few days and 2 weeks after the oilseed rape had been sown (Table 1), suggesting that plants in uncultivated habitats near treated crops can be an additional source for pesticide exposure[26].

We draw two main conclusions from our study. First, clothianidin seed coating in oilseed rape has negative effects on wild bees, with potential negative effects on populations. This finding is important because of the urgency to understand whether the use of neonicotinoid insecticides pose an unacceptable risk to bees[6]. However, questions remain regarding the mechanisms by which neonicotinoids affect bees, how field exposure varies across crops and seasons, and if effects translate into long-term population consequences, which are the focus of our further research. Second, the impact of clothianidin seed coating in oilseed rape differs between the wild bees studied and the honeybee. This implies that the use of honeybees as model organisms[27] in environmental risk assessments of neonicotinoids may not allow generalizations to other bee species. We question whether prevailing risk assessment standards, where predominantly short-term and lethal effects are assessed on model species under laboratory conditions[27,28], can be used to predict real-world consequences of pesticide use for populations, communities and ecosystems[29,30].

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions R.B., I.F., T.R.P. and H.G.S. conceived the project. M.R. designed the study, coordinated the work, analysed the data, and prepared the manuscript. G.K.S.A., V.H., L.H., B.K.K. and J.Y. collected the data. O.J. quantified the pesticide residues. All authors contributed to the interpretation of results and writing of the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.R. (maj.rundlof@biol.lu.se).

METHODS

Study design. The design initially included 20 fields matched into pairs based on land use within 2 km (Extended Data Table 1), to cover the foraging distance of most bees[31,32], and geographical proximity. One field in each pair was randomly assigned to be sown with insecticide-coated seeds and the other field was the control field. The matching into field pairs was based on available land-use data for 2011, and the landscapes surrounding the selected oilseed rape fields were inspected for presence of flowering crops (including other spring-sown oilseed rape fields) during 27–28 May 2013. At the same time, establishment and growth stages (using the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie)[33]) of the oilseed rape plants in the focal fields were inspected. After the field inspections, three fields were excluded from the study, because there were four (total 20.9 ha), five (22.6 ha) or five (46.2 ha) additional spring-sown oilseed rape fields within 2 km from our focal field that, since our study was conducted before the moratorium[6], may have been additional sources of neonicotinoid exposure. One field was excluded because a red clover seed field, known to be very attractive to foraging bumblebees and influence their density in the surrounding landscape[19], occurred adjacent to the focal field. In two cases, we decided to accept a single other oilseed rape field located at distances of 0.9 km (6.5 ha) and 1.0 km (4.4 ha) from the planned location of our bee colonies, to retain as many replications of fields as possible. At this point, the study design included six original field pairs and four fields which had lost their pair field. After reviewing land use in the surrounding landscapes and in geographical proximity of the four unpaired fields, we decided to match these into two new pairs (P07 and P08 in Fig. 1). The final study system included 16 spatially separated (>4 km) spring-sown oilseed rape fields (mean ± s.e.m. field size 8.9 ± 1.4 ha, range 4–27 ha, with all fields but one control field in the range 4–11 ha) located in southern Sweden (Fig. 1). The landscapes surrounding the fields were distributed along a gradient in the proportion of agricultural land, ranging from 6–88%, and the land uses considered often co-varied (Extended Data Table 1).

The field in each pair that was randomly assigned to be sown with insecticide-coated seeds received seeds treated with 25 ml Elado (Bayer; 400 g l^{-1} clothianidin + 80 g l^{-1} β-cyfluthrin) per kg of seed and the fungicide thiram, and the other field in the pair was sown with seeds coated with only thiram (the control). Elado instead of only clothianidin was used, because the pesticide combination was an agronomically realistic scenario for clothianidin use in Sweden and in other parts of Europe[34]. The clothianidin is taken up by the plant, distributed to all parts and protects the whole plant from pest attack[5], while the non-systemic β-cyfluthrin is intended to protect seeds and roots and only a very small amount is found in the aboveground parts of the plant (<0.5% of applied)[18]. We did not detect any β-cyfluthrin in pollen collected by honeybees at fields with insecticide seed coating (Extended Data Table 8). Fungicides alongside neonicotinoids have frequently been used in coating oilseed rape seeds (A. Gunnarson, personal communication)[35,36]. Since our study was conducted before the moratorium[6], no approval for the use of clothianidin-dressed seeds had to be obtained.

All experimental fields were sown with the hybrid oilseed rape cultivar Majong. The amount of seeds sown was 150 plants per square metre, which is the recommended seeding rate for a spring-sown hybrid[37], and corresponds to 7.5 kg ha^{-1} for thiram-treated seeds and 7.7 kg ha^{-1} for Elado + thiram-treated seeds. Sowing time was chosen and carried out by each farmer during the period 6 April to 18 May 2013 (Extended Data Table 2). In two of the pairs, the treated fields were sown considerably earlier (both 21 April) than the control fields (6 and 7–8 May), resulting in a phenological asynchrony between the fields in these pairs.

Farmers were not allowed to use other neonicotinoids in the fields, but they could use the non-neonicotinoid compounds Avaunt (active ingredient: indoxacarb), Mavrik (active ingredient: τ-fluvalinate), Plenum (active ingredient: pymetrozine) and Steward (active ingredient: indoxacarb) to control pollen beetles (Extended Data Table 3). Nevertheless, in one case, at a control field, the farmer applied Biscaya (Extended Data Table 3), where the active ingredient is the neonicotinoid thiacloprid. Thiacloprid has lower acute toxicity for bees than clothianidin, imidacloprid or thiamethoxam[13,22,25] and excluding this field did not qualitatively influence the effect of the insecticide seed treatment on the bees (Extended Data Tables 4–6).

Wild bee monitoring. Wild bees and flower cover were surveyed on three occasions in the flowering oilseed rape fields and adjacent uncultivated field borders, between 17 June and 16 July 2013 (Extended Data Table 2). Four in-field transects of 2 × 25 m located 2–4 m from the edge of the oilseed rape field were surveyed twice (18 June to 12 July and 27 June to 16 July). Transects of 2 × 300 m located at the outer 1-m edge of the oilseed rape field and 1 m of the adjacent, uncultivated field border were surveyed once (17 June to 8 July). Border transects within a field pair were surveyed on the same or subsequent days for the six phenologically synchronous field pairs and at peak flowering at the fields in the two asynchronous field pairs. For in-field transects, at least one of the survey occasions was performed within subsequent days for fields within a pair for all pairs but one (P04), and the other survey at peak flowering within the individual fields (Extended Data Table 2). Surveys were only conducted on warm days with no rain and light winds (<7 m s^{-1}). The observer covered approximately 10 m^2 of transect per minute. All flower visiting and flying solitary bees and bumblebees within the transects were noted and determined to species, genera or taxonomic group (Extended Data Table 7), using the entomological collection at Lund University, and refs 38, 39 and 40. Bumblebees belonging to the *B. terrestris* complex, including *B. terrestris*, *Bombus lucorum*, *Bombus magnus* and *Bombus cryptarum*, could not be separated and were treated as one group (*B. terrestris* ag.). Flower cover was calculated based on measurements of the number and size of flowers within transects.

Solitary bee nesting. Three trap nests (CJ Wildlife), each containing 29 paper tubes with an inner diameter of 6 mm and nine *O. bicornis* (previously *Osmia rufa*) cocoons (four females and five males), in total 27 cocoons (12 females and 15 males) were placed at each field approximately a week before the latest field within a pair was estimated to start flowering (equivalent to stage 55–63 on the BBCH scale, where stage 55 corresponds to individual buds being visible but still closed and stage 63 corresponds to the time when 30% of the flowers on the main raceme has opened[33]), between 10 and 24 June 2013 (Extended Data Fig. 1 and Extended Data Table 2). After emergence from the cocoons, *O. bicornis* individuals mate and the female starts to build cells where she places her eggs on collected pollen[41]. Emergence was the same in both treatments (Extended Data Fig. 1a). Females prefer to return to and build cells in their natal nest, over new equivalent nest cavities[42,43], and there is indication that nest site availability is limiting populations in current agricultural landscapes[44].

The cocoons originated from the study region. We artificially delayed emergence by about a month, by storing cocoons at 2–5 °C, to match the phenology of the spring-sown oilseed rape. In our study region in southern Sweden, observations of the species since 1990 indicate May (255 observations) to be the main activity period of *O. bicornis*, followed by April (94), June (83), July (2) and March (1)[45] (access date 9 February 2014, species: "*Osmia bicornis*", region: "Götaland", period: "1990-2014", "March", "April", "May", "June", "July" and "August"). This indicates that most of the *O. bicornis* at our study fields likely originated from the introduced population. Placement at the fields occurred on the same day at fields within a pair (Extended Data Table 2). Trap nests were mounted on poles in the field borders, approximately 50 m apart, facing southwards and with sheltering vegetation at the northern side.

Nesting tubes were collected 36–43 days after installing the cocoons. Nesting activity was determined in October 2013 by counting the number of tubes with brood cells. Where nesting activity occurred, *O. bicornis* built 4–34 brood cells in total per field (3.5 ± 0.3 (mean ± s.e.m.) cells per nest and field), distributed over 1–9 tubes. Proportion emerging from the cocoons was determined by counting the number of open cocoons. The pupa was considered dead if the cocoon was intact 4 weeks after placement at the fields.

Bumblebee colonies. Six commercially reared *Bombus terrestris* colonies (Natupol N, Koppert Biological Systems) were placed at each field at the onset of oilseed rape flowering, between 14 and 28 June 2013. At this time, the colonies were approximately 10 weeks old and contained one queen, approximately 50 workers and brood in all stages. Placement followed the phenology of the oilseed rape fields and was done on the same day in six of the pairs (or 2 days apart in one case) for fields within a pair (Extended Data Table 2). Placements of colonies at the two field pairs with asynchronous phenology were separated by 8 days between fields within the pairs, to follow the onset of flowering in the individual fields (Extended Data Table 2). Bumblebee colonies were ordered in four batches, with colonies from the same batch in the six synchronous pairs and from batches matching the individual fields for the two asynchronous pairs (Extended Data Table 2). Prevalence of pathogens and parasites in the colonies were not quantified before placement, although commercial colonies can be infested[46], and this could add unexplained noise to our data. Colonies were placed in triplets in two ventilated houses, located in a shaded part of the field borders (Extended Data Fig. 1). The colonies did not receive any supplementary feeding after placement at the fields. The inner plastic boxes and the *B. terrestris* colony content (bees, brood and nesting material) were weighed when placed at the fields and thereafter approximately biweekly. Colonies were closed for exiting bees before weighing and each colony was weighed 3–5 times (including the initial weighing). Two colonies (one at each treatment) were not weighed at one occasion, because they could not be closed for exiting bees. All colonies within a field pair were terminated by freezing (−20 °C) at first sight of emerging new queens in any of the 12 colonies. This happened between 7 July and 5 August 2013, or 23–38 days after the colonies had been placed at the fields. At the asynchronous field pairs, the colonies were collected at different dates from fields within the pair, but were allowed an equal number of days from placement to termination.

The outer two colonies in each triplet box were examined to estimate the number of queen and worker/male cocoons, weight of cocoons, larvae and nest structure and the number of cells used for nectar and pollen storage. Separation between queen and worker/male cocoons were based on the lowest value between the peaks of the bimodal distribution of cocoon width, based on measurement of all cocoons from four of the colonies (Extended Data Fig. 1c).

Honeybee colonies. Six equally sized *Apis mellifera* colonies were placed at each field (in total 96 colonies) at the onset of oilseed rape flowering, on 14–28 June 2013 (Extended Data Table 2), containing an estimated 3,418 ± 123 (mean ± s.e.m.) adult bees per colony (with no statistical difference between treatments (Extended Data Table 5)). Placement at the fields followed their phenology and was done on the same day (or two days apart in one case) for fields in six of the pairs (Extended Data Table 2). At the two field pairs with asynchronous phenology, placements were separated by seven days between fields within the pairs, following the onset of flowering in the individual fields (Extended Data Table 2).

Honeybee colony strength (that is, number of adult bees per colony) was assessed before placement at the experimental fields, on 6–7 June, and again at a common over-wintering location after removal from the experimental fields, on 29 July to 2 August, by a trained observer using the Liebefeld method[47,48]. The colonies were removed from the experimental fields on 2–31 July, at the end of oilseed rape flowering.

The colonies were produced on 27–31 May by a professional beekeeper with 1- or 2-year-old queens of known descent. Colonies were equalized to include two full honeycombs (with bees), two combs with mainly sealed brood (with bees), one queen originating from the same colony as the one from which the split (newly created colony) was taken, bees from two combs shaken into the split, one drawn out empty comb and five combs with wax foundation. The queens in the splits were freely mated and derived from three different mother queens and consisted of four different groups based on queen lineage and age. Queen lineage and age were matched between fields within a pair, but the distribution of colonies was otherwise randomized. The comb size was full Langstroth, with an area of 880 cm^2 per comb side and an estimated 1.25 bees per cm^2 when a comb side was fully covered (a total of 1,100 bees per side)[49]. Parent colonies and the new splits were placed in a 60 ha field of organically grown winter-sown oilseed rape after equalization and before placement at the 16 experimental fields, to minimize the risk of exposure to clothianidin and other pesticides.

Pollen samples. To verify the use of oilseed rape by the bees, pollen samples were taken from pollen traps mounted on the *A. mellifera* colonies, from *B. terrestris* foraging in the fields and from *O. bicornis* brood cells. The pollen traps were mounted on three *A. mellifera* colonies and were activated during the peak flowering of the oilseed rape (stages 65–67 on the BBCH scale[33]). At least 25 ml of pollen was collected from each field. A subsample of 15.0 g of the *A. mellifera*-collected pollen was sorted into separate samples based on colour and the separate samples were weighted. One to five samples from *B. terrestris* were collected per field (2.9 ± 0.3 (mean ± s.e.m.)), giving a total of 47 samples. Pollen was collected, when possible, from *O. bicornis* larval cells, resulting in 17 samples from the six control fields with nesting activity.

50–500 random pollen grains per sample were determined to have originated from either oilseed rape or another plant species using microscopy (10–40× magnification) and the pollen reference collection at Department of Biology, Lund University.

Neonicotinoid residues. Vegetation, pollen and nectar samples were collected to quantify the concentrations of clothianidin, together with β-cyfluthrin and the other four neonicotinoids used in Sweden (Extended Data Table 8), and to confirm the treatments. Samples of herbaceous material (flowers and leaves) were collected, within 2 days of sowing (7 April–20 May), every tenth metre in the transects used for wild bee monitoring in the permanent field borders adjacent to the oilseed rape fields. At the treated fields we also collected similar vegetation samples 13–15 days after sowing (21 April–3 June). In each field, five *A. mellifera* with pollen loads were caught to collect pollen samples and at least five nectar foragers were caught to collect nectar from the honey stomach. At two of the control fields, no *A. mellifera* with pollen loads could be found in the oilseed rape fields. Five *B. terrestris* were caught in the flowering oilseed rape fields, brought to the laboratory and nectar was extracted from the nectar stomachs of 3–5 bees per field, except at one control field where only one bee carried nectar.

Nectar samples were quantitatively handled using the capillary microsampling technique[50–52]. Neonicotinoids were quantified using liquid chromatography coupled with tandem mass spectrometry. β-Cyfluthrin was quantified using gas chromatography coupled with mass spectrometry. See Extended Data Table 8 for limits of detection and quantification.

Observer blind data collection. The people monitoring wild bees in the oilseed rape fields, handling the solitary bee nests, weighing and examining the bumblebee colonies, assessing the honeybee colony strength, and collecting honeybee pollen and nectar samples were blinded with respect to treatment. However, for practical reasons it was not possible to blind the person collecting vegetation samples in field borders during sowing and thereafter monitoring wild bees in the border transects and collecting bumblebees for pollen and nectar samples.

Statistical analyses. All data was analysed in SAS 9.4 for Windows (SAS Institute Inc.).

Wild bee densities were compared between treatments and in relation to flower cover, size of the focal oilseed rape field and proportion of agricultural land in the surrounding landscape using a generalized linear mixed model (GLMM, SAS PROC GLIMMIX) with Poisson error distribution and log link. Pair identity, pair identity × treatment and field part nested within pair identity × treatment were included as random factors, to account for the pairing of sites and the hierarchical study design. To investigate if the difference in phenology between fields influenced the difference in wild bee density between treatments, we also ran a statistical model only including temporally synchronous surveys, that is, surveys not more than 1 day apart for fields within a pair (Extended Data Table 2). In addition, to investigate if the influence of treatment was consistent for strictly wild bees, we ran another two models, but excluded *B. terrestris* ag., which could originate from the commercial colonies, and all bumblebees not determined to species (Extended Data Table 7). Results from all four analyses were qualitatively the same, except for flower cover, which did not relate significantly to the strictly wild bee density (Extended Data Table 4). GLMM with binomial error distribution and logit link were used to test the difference in flower cover between treatments, both for all data and for only temporally synchronous surveys (Extended Data Table 5). Results did not differ qualitatively depending on data set used (Extended Data Table 5).

Differences in emergence of *O. bicornis* from the cocoons between treatments, sexes and their interaction were tested with a GLMM with binomial error distribution and logit link. Pair identity, pair identity × treatment and sex nested within pair identity × treatment were included as random factors. The number of *O. bicornis* nest tubes with nesting activity was compared between treatments using Wilcoxon–Mann–Whitney test (SAS PROC NPAR1WAY).

An individual growth model (Extended Data Table 6) based on a linear mixed model (LMM, SAS PROC MIXED)[53] was used to test the effect of treatments on the weight gain of the *B. terrestris* colonies from placement at the fields (day = 0). The net weight gain was related to day, treatment, day × treatment, day × day and day × day × treatment. Random intercepts and random slopes for day and day × day were included, with the colony identity as the subject and an unstructured covariance matrix. Pair identity and pair identity × treatment were included as random factors to account for the study design. Since the individual growth model was complex and yielded significant two- and three-way interactions between treatment, we decided to: (1) analyse growth over time separate for the two treatments (Extended Data Table 6); and (2) test differences in colony growth rate between treatments only for the positive growth phase, identified as the period until the peak weight at control fields, using a LMM with estimated slope as the dependent variable, treatment as the independent variable and pair identity as a random factor. LMM (with normal error distribution) or GLMM (with Poisson error distribution and log link) were used to compare the number of queen and worker/male cocoons, weight of cocoons, larvae and nest structure and the number of cells used for nectar and pollen storage between treatments (Extended Data Table 5).

Honeybee colony strength (that is, number of adult bees per colony) was compared between treatments using a LMM. Colony strength before placement at the fields was used as a covariate and pair identity and pair identity × treatment were included as random factors. A colony that lost its queen during transport to the field (treated field) and swarmed colonies (eight at control fields and ten at treated field) were excluded from the analysis (which did not qualitatively alter the results).

To investigate if the presence of other spring oilseed rape fields within 1 km influenced the results, *B. terrestris* colony growth (Extended Data Table 5), *B. terrestris* queen and worker/male production (Extended Data Table 5) and *A. mellifera* colony development (Extended Data Table 6) were analysed using the full data set as well as a data set where the two field pairs with other spring-sown oilseed rape within 1 km from one of the fields were excluded, since the other spring-sown oilseed rape fields were within the potential flight range of both bee species[31,32]. The results were qualitatively the same for *B. terrestris* colony growth, weight of produced cocoons and *A. mellifera* colony development independent of including or excluding the two field pairs (Extended Data Tables 5 and 6), but differed for the number of *B. terrestris* cocoons (Extended Data Table 5). The latter could be a result of reduced statistical power to detect differences, since the level of replication is reduced from eight to six when excluding two of the field pairs and queen production in particular is documented to be very variable[10,54–56].

To investigate if the Biscaya used at one control field influenced the results, we analysed wild bee density (Extended Data Table 4), *O. bicornis* nesting activity (results not shown), *B. terrestris* colony growth (Extended Data Table 5), *B. terrestris* queen and worker/male production (Extended Data Table 5) and *A. mellifera* colony development (Extended Data Table 6) in relation to the insecticide seed treatment both including and excluding the field pair where Biscaya was used at the control field. The results were qualitatively the same for all dependent variables independent of including or excluding the field pair (Extended Data Tables 4–6).

The clothianidin concentrations in nectar and pollen collected by honeybees, nectar collected by bumblebees and field border plant material were analysed in relation to treatment using Wilcoxon–Mann–Whitney tests.

Denominator degrees of freedom in the mixed models were estimated with the Kenward–Roger method or, when there was a negative covariance in the random part of the model, the containment method (constraining the variance component to 0), to avoid inflated denominator degrees of freedom[53]. Deviance from the assumption of normal error distribution of the LMM was tested using a Shapiro–Wilks test and visually assessed on residual plots. When deviance was detected ($P < 0.05$ and indicated in plots), data was either square-root transformed or a GLMM, assuming Poisson error distributions, was used. Deviance from the assumption of homogeneous variance between compared groups was tested using Levene's test. When deviance was detected ($P < 0.05$), heterogeneous variance was modelled. Over-dispersion of the data, when the variance is considerably larger than the mean, was assessed by the ratio of the generalized χ^2 statistics and its degrees of freedom[53]. If the ratio was larger than 1.3, an over-dispersion parameter (random _residual_) was added to the model.

Power analysis. We performed a power analysis for honeybee colony strength, to investigate the effect size that we could potentially detect given our design and replication. A power analysis is conditional on the study design and the statistical model used to analyse the data, so we therefore used a power analysis method recommended for mixed models[53]. With the macro (program) MixedTPower[53] we produced a power curve based on the honeybee colony strength model. We assumed $\alpha = 0.05$ and then calculated power for a range of effect sizes. The effect size is initially expressed on the same scale as the dependent variable (that is, number of bees per colony; Extended Data Fig. 2a). By dividing the effect size with the average number of bees per colony at control sites, we obtained effect size expressed as the percentage change in the number of bees per colony (that is, colony strength) between control fields and treated fields (Extended Data Fig. 2b), which made it possible to compare our effect size with the effect sizes stated by the European Food Safety Authority [57] and the power analysis performed by the Centre for Ecology and Hydrology [58]. Our power analysis indicated that, given our design, replication and data analysis method, we would be able to detect an effect size of just below 20% with a power of 0.8 (Extended Data Fig. 2b). This is in line with the estimated effect size for our level of replication given by the Centre for Ecology and Hydrology [58].

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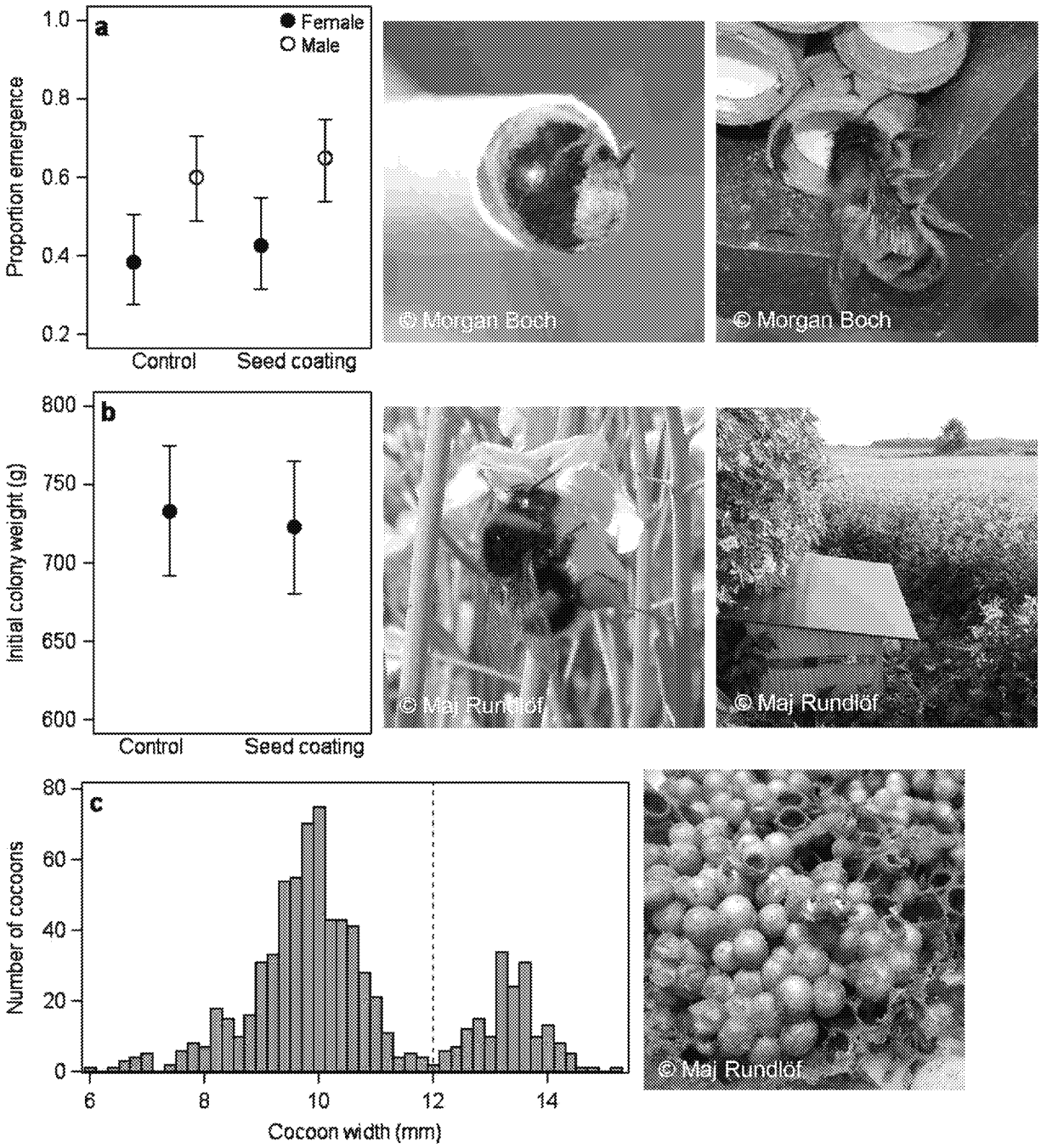
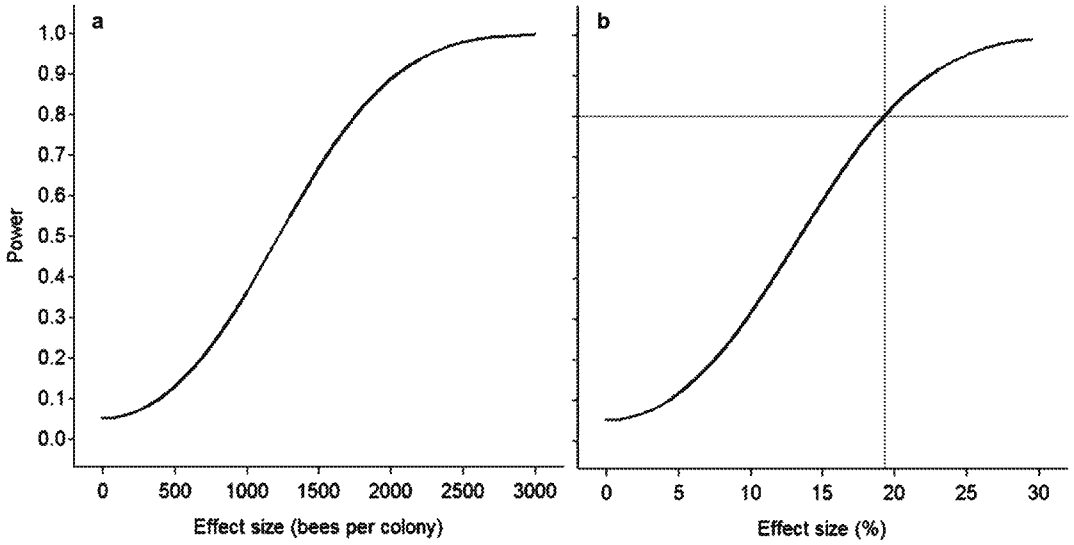


Extended Data Figure 1 | *O. bicornis* emergence and *B. terrestris* colonies. **a**, Mean (± 95% confidence limits) proportion emergence of *O. bicornis* from cocoons in relation to treatment (control or insecticide seed coating), with higher emergence for males than females (generalized linear mixed model, binomial error distribution, logit link; $F_{1,14} = 14.97$, $P = 0.0017$), no difference between treatments ($F_{1,7} = 0.71$, $P = 0.43$) and no interaction ($F_{1,14} = 0.01$, $P = 0.94$). $n = 8$ fields per treatment, with 12 female and 15 male cocoons at each field. Photos (with permission; Morgan Boch): left, emerged *O. bicornis* cocoon; right, *O. bicornis* female at a trap nests filled with cardboard nest tubes. **b**, Mean (± 95% confidence limits) weight of *B. terrestris* colonies at placement at the fields in relation to treatment (linear mixed model, $F_{1,7} = 0.99$, $P = 0.35$). $n = 8$ fields per treatment, with six colonies at each field. Photos (M.R.): left, *B. terrestris* worker foraging in the oilseed rape; right, house containing three *B. terrestris* colonies. Means and confidence limits in panels **a** and **b** are based on back-transformed, model-estimated least square means. **c**, *B. terrestris* silk cocoon width distribution of all cocoons in four colonies (two from two different control fields and two from two different fields with insecticide seed treatment) initially examined to separate between queen and worker/male cocoons. Dashed vertical line indicates selected cut-off width at 12 mm (the lowest value between the two peaks), with queens larger (or equal) and workers/males smaller. Photo (M.R.): *B. terrestris* colony under examination.



Extended Data Figure 2 | Power curves for honeybee colony strength. **a**, **b**, Relationship between power and effect size estimated for the honeybee model (Extended Data Table 6), with effect size expressed as the difference in honeybee colony strength (number of bees per colony) (**a**) and the percentage change in colony strength (**b**) between colonies at control fields and at fields with insecticide seed coating after placement at the oilseed rape fields. Grey reference lines indicate a power of 0.8 and the corresponding effect size.

Extended Data Table 1 | 2013 field size and 2011 and 2013 land use in the landscapes surrounding (radius = 2 km) the oilseed rape

	Control (n = 8)		Insecticide seed coating (n = 8)		Test of difference between treatments		Correlation matrix									
	mean ± s.e.m.	min-max	mean ± s.e.m.	min-max	F_{df}	P	Agricultural land	Annually tilled arable land	Semi-natural grassland	Length of permanent field borders	Maize cultivation	Spring sown oilseed rape	Winter sown oilseed rape	Mass-flowering crops*	Forest	Urban
Size of focal oilseed rape field (ha)	9.4 ± 2.6	4.0-27.0	8.4 ± 0.9	4.0-11.0	$0.11_{1,7}$	0.75	0.102	0.252	-0.425	0.033	-0.130	-0.174	-0.048	0.543	-0.159	0.300
Agricultural land (%)	58.2 ± 10.6	9.5-88.2	55.8 ± 9.8	5.9-83.3	$0.29_{1,7}$	0.61		0.923	-0.049	0.831	0.401	0.309	0.539	0.744	-0.962	-0.083
Annually tilled arable land (%)	38.8 ± 9.6	3.0-70.9	34.3 ± 8.8	0.3-74.5	$0.64_{1,7}$	0.45			-0.338	0.592	0.173	0.334	0.675	0.870	-0.866	-0.069
Semi-natural grassland (%)	3.1 ± 1.0	0.2-7.4	4.1 ± 1.2	0.2-9.4	$0.16_{1,7}$	0.70				0.381	0.450	-0.259	-0.285	-0.337	0.082	-0.096
Length of permanent field borders (km)	14.2 ± 1.9	3.5-18.5	14.9 ± 2.3	3.2-25.7	$0.11_{1,7}$	0.75					0.688	0.157	0.139	0.462	-0.827	0.009
Maize cultivation 2011 (%)	1.4 ± 0.5	0-3.9	1.7 ± 0.8	0-6.5	$0.02_{1,7}$	0.88						0.272	-0.243	0.107	-0.483	0.203
Maize cultivation 2013 (%)	1.3 ± 0.4	0-3.6	1.3 ± 0.7	0-5.6	$0.42_{1,7}$	0.54										
Spring sown oilseed rape 2011 (%)	0.8 ± 0.7	0-5.7	0.6 ± 0.2	0-1.5	$0.05_{1,7}$	0.83							-0.137	0.785	-0.254	-0.217
Spring sown oilseed rape 2013 (%) – including the focal field	1.8 ± 0.7	0.3-6.2	1.5 ± 0.4	0.3-2.7	$<0.01_{1,7}$	0.98										
Winter sown oilseed rape 2011 (%)	1.4 ± 0.8	0-6.8	1.6 ± 1.0	0-8.2	$<0.01_{1,7}$	0.96								0.566	-0.455	-0.163
Winter sown oilseed rape 2013 (%)	1.5 ± 0.7	0-5.2	2.5 ± 1.2	0-8.6	$0.34_{1,7}$	0.58										
Mass-flowering crops* 2013 (%)	8.2 ± 2.8	0.3-23.6	7.5 ± 2.1	0.8-17.8	$0.01_{1,7}$	0.93										
Forest (%)	25.3 ± 10.6	1.8-74.8	24.0 ± 8.6	0.5-67.2	$0.23_{1,7}$	0.64								-0.693		-0.116
Urban (%)	2.7 ± 1.1	0-8.6	3.3 ± 1.0	0-9.0	$0.53_{1,7}$	0.49								0.026		

*Mass-flowering crops include oilseed rape (46%), potato (28%), pea (18%), bean (4%), fruit and berry cultivation (4%), and herbs and seeds (<1%).

Extended Data Table 2 | Phenology (date, BBCH[33] and flower cover) in the oilseed rape fields and delivery, placement and survey* of bees

Pair	Seed treatment†	Sowing date	Date placement *Osmia bicornis* (oilseed rape growth stage (BBCH))	*Bombus terrestris* delivery date (batch)	Date placement *Bombus terrestris* (oilseed rape growth stage (BBCH))	Date placement *Apis mellifera* (oilseed rape growth stage (BBCH))	Date wild bee survey border (oilseed rape growth stage (BBCH))	Date wild bee survey field 1 (% flower cover)	Date wild bee survey field 2 (% flower cover)
P01	contr	23 April 2013	13 June (59)	18 June (2)	20 June (65)	19 June (65)	25 June (65)	1 July (52)	3 July (43)
P01	treat	28 April 2013	13 June (57)	18 June (2)	20 June (61)	19 June (61)	26 June (63)	1 July (95)	3 July (97)
P02	contr	7-8 May 2013‡	13 June (50)	20 June (3)	26 June (63)	25 June (63)	6 July (65)	28 June (58)	9 July (60)
P02	treat	21 April 2013‡	13 June (61)	18 June (2)	18 June (63)	18 June (63)	20 June (63)	19 June (90)	27 June (49)
P03	contr	18 May 2013	24 June (52)	25 June (4)	28 June (60)	2 July (61)	8 July (63)	12 July (33)	16 July (46)
P03	treat	11 May 2013	24 June (57)	25 June (4)	28 June (61)	2 July (63)	8 July (65)	8 July (53)	12 July (64)
P04	contr	6 May 2013‡	13 June (50)	20 June (3)	26 June (65)	25 June (65)	4 July (65)	7 July (56)	9 July (61)
P04	treat	21 April 2013‡	13 June (61)	18 June (2)	18 June (63)	18 June (63)	20 June (65)	19 June (89)	1 July (37)
P05	contr	29 April 2013	15 June (57)	18 June (2)	20 June (63)	20 June (63)	24 June (65)	24 June (21)	4 July (39)
P05	treat	25 April 2013	15 June (61)	18 June (2)	18 June (63)	18 June (63)	24 June (65)	24 June (57)	4 July (100)
P06	contr	1 May 2013	13 June (57)	18 June (2)	19 June (63)	19 June (63)	28 June (65)	2 July (74)	5 July (94)
P06	treat	25-26 April 2013	13 June (53)	18 June (2)	19 June (63)	19 June (63)	28 June (65)	5 July (89)	9 July (81)
P07	contr	4 May 2013	15 June (55)	20 June (3)	24 June (63)	24 June (63)	1 July (65)	7 July (26)	11 July (33)
P07	treat	2 May 2013	15 June (57)	20 June (3)	24 June (64)	24 June (64)	1 July (65)	7 July (87)	11 July (39)
P08	contr	6 April 2013	10 June (61)	11 June (1)	14 June (65)	14 June (65)	17 June (65)	18 June (43)	28 June (5)
P08	treat	16 April 2013	10 June (61)	11 June (1)	14 June (63)	14 June (63)	18 June (65)	18 June (14)	28 June (72)

*Shaded numbers are surveys selected for analysis of wild bee density data collected at the same time (that is, within subsequent days) within the field pairs.
†contr, control; treat, insecticide seed coating.
‡Highly asynchronous phenology of the fields within the pair.

Extended Data Table 3 | Use of plant protection products in the oilseed rape fields during the 2013 growing season

Pair	Seed treatment*	Date treatment 1	Compound treatment 1	Dose treatment 1	Date treatment 2	Compound treatment 2	Dose treatment 2
P01	contr	04 June 2013	Mavrik	0.25 l/ha			
P01	treat	06 June 2013	Plenum	150 g/ha	15 June 2013	Steward	85 g/ha
P02	contr	31 May 2013	Plenum	160 g/ha	10 June 2013	Mavrik	0.20 l/ha
P02	treat	04 June 2014	Plenum	150 g/ha	10 June 2013	Steward	85 g/ha
P03	contr	no treatment					
P03	treat	12 June 2013	Avaunt	170 g/ha			
P04	contr	16 June 2013	Avaunt	160 g/ha			
P04	treat	07 June 2013	Plenum	150 g/ha			
P05	contr	12 June 2013	Plenum	150 g/ha			
P05	treat	30 May 2013	Plenum	150 g/ha			
P06	contr	12 June 2013	Biscaya	0.30 l/ha	19 June 2013	Mavrik	0.25 l/ha
P06	treat	07 June 2013	Avaunt	170 g/ha			
P07	contr	04 June 2013	Avaunt	170 g/ha	08 June 2013	Plenum	150 g/ha
P07	treat	31 May 2013	Plenum	150 g/ha			
P08	contr	30 May 2013	Avaunt	170 g/ha			
P08	treat	04 June 2014	Plenum	150 g/ha	14 June 2013	Avaunt	120 g/ha

*contr, control; treat, insecticide seed coating.

Extended Data Table 4 | Wild bee density in oilseed rape fields and borders in relation to insecticide seed treatment and covariates

Model	Explanatory variable	Estimate	Degrees of freedom	*F*	*P*
Wild bees (all data)	Intercept	2.55			
	Treatment	0.73	1, 7	9.68	0.019
	Flower cover	1.06	1, 24	18.57	<0.001
	Field size	0.07	1, 7	7.46	0.028
	Proportion agricultural land	-1.20	1, 8	2.35	0.16
Wild bees (synchronized data*)	Intercept	2.03			
	Treatment	0.76	1, 6	6.69	0.043
	Flower cover	1.32	1, 29	26.56	<0.001
	Field size	0.08	1, 7	6.46	0.038
	Proportion agricultural land	-1.00	1, 5	2.76	0.15
Wild bees excluding *Bombus terrestris* ag. (all data)	Intercept	0.79			
	Treatment	1.14	1, 7	12.65	0.0096
	Flower cover	1.06	1, 17	8.52	0.094
	Field size	0.08	1, 6	6.63	0.045
	Proportion agricultural land	-0.33	1, 7	0.20	0.67
Wild bees excluding *Bombus terrestris* ag. (synchronized data*)	Intercept	-16.07			
	Treatment	9.16	1, 4	12.28	0.025
	Flower cover	2.17	1, 7	0.35	0.57
	Field size	1.77	1, 7	54.65	<0.001
	Proportion agricultural land	4.86	1, 7	1.07	0.34
Wild bees (excluding the field pair where Biscaya was used at the control field)	Intercept	0.93			
	Treatment	0.95	1, 3	20.20	0.023
	Flower cover	1.18	1, 15	16.29	0.0011
	Field size	0.20	1, 4	10.04	0.034
	Proportion agricultural land	-0.42	1, 8	0.12	0.74

*See Extended Data Table 2 for identification of synchronized data.

Extended Data Table 5 | Statistical tests and mean values for bee-related variables in relation to the insecticide seed treatment in the oilseed rape fields

Dependent variable	Degrees of freedom	*F*	*P*	Control (mean ± s.e.m.)	Insecticide seed coating (mean ± s.e.m.)
Flower cover (%) - all data	1, 7	9.34	0.018	46.4 ± 7.3	70.2 ± 6.5
Flower cover (%) - synchronized data*	1, 6	8.28	0.028	41.4 ± 9.0	70.9 ± 8.0
Initial *Bombus terrestris* colony weight (g)	1, 7	0.99	0.35	733.2 ± 17.8	722.7 ± 18.6
Slope of *Bombus terrestris* colony growth	1, 7	115.80	<0.001	21.3 ± 1.6	0.4 ± 1.6
Slope of *Bombus terrestris* colony growth - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	143.02	<0.001	18.9 ± 1.1	-0.5 ± 1.1
Slope of *Bombus terrestris* colony growth - excluding the field pair where Biscaya was used at the control field	1, 6	108.41	<0.001	22.2 ± 1.7	0.5 ± 1.7
Number of *Bombus terrestris* queen cocoons	1, 7	7.78	0.027	70.0 ± 12.3	20.6 ± 8.3
Number of queen cocoons - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	3.82	0.11	59.7 ± 15.8	22.0 ± 9.6
Number of queen cocoons - excluding the field pair where Biscaya was used at the control field	1, 6	9.46	0.022	69.1 ± 13.7	18.1 ± 7.0
Number of *Bombus terrestris* worker/male cocoons	1, 7	8.09	0.025	241.0 ± 29.8	142.0 ± 29.8
Number of worker/male cocoons - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	6.57	0.050	206.1 ± 28.3	115.6 ± 20.7
Number of worker/male cocoons - excluding the field pair where Biscaya was used at the control field	1, 6	6.74	0.041	247.6 ± 33.9	144.0 ± 33.9
Weight of *Bombus terrestris* cocoons (g)	1, 7	14.77	0.0061	172.0 ± 32.3	54.0 ± 18.7
Weight of cocoons (g) - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	12.34	0.017	135.1 ± 25.3	41.6 ± 14.5
Weight of cocoons (g) - excluding the field pair where Biscaya was used at the control field	1, 6	9.62	0.021	201.1 ± 32.3	69.2 ± 32.3
Weight of *Bombus terrestris* larvae (g)	1, 7	0.15	0.71	15.5 ± 6.0	13.6 ± 5.7
Weight of *Bombus terrestris* nest structure (g)	1, 7	12.34	0.0098	261.0 ± 24.7	139.4 ± 24.7
Number of nectar cells	1, 7	2.43	0.16	59.4 ± 23.7	23.5 ± 10.4
Number of pollen cells	1, 7	0.60	0.46	5.5 ± 2.1	3.6 ± 1.4
Initial number of *Apis mellifera* per colony	1, 7	0.12	0.74	3412 ± 192	3325 ± 160
Proportion oilseed rape pollen from *Osmia bicornis* (%)				35.1 ± 17.0	
Proportion oilseed rape pollen from *Bombus terrestris* (%)	1, 8	3.70	0.092	88.1 ± 5.0	74.9 ± 7.7
Proportion oilseed rape pollen from *Apis mellifera* (%)	1, 7	1.09	0.33	52.6 ± 7.2	63.1 ± 6.9

*See Extended Data Table 2 for identification of synchronized data.

Extended Data Table 6 | Bumblebee colony growth (net weight gain) and honeybee colony strength (adult bees per hive) in relation to insecticide seed treatment

Model	Explanatory variable(s)	Estimate	Degrees of freedom	*F*	*P*
***B. terrestris* colony growth**					
All fields	Intercept	-51.07			
	Treatment	-434.27	1, 18	51.41	<0.001
	Day	0.23	1, 21	144.31	<0.001
	Day × treatment	72.50	1, 21	143.00	<0.001
	Day × day	0.08	1, 19	102.52	<0.001
	Day × day × treatment	-1.40	1, 19	130.62	<0.001
Only control fields	Intercept	-533.40			
	Day	77.59	1, 31	129.10	<0.001
	Day × day	-1.44	1, 28	114.70	<0.001
Only fields with insecticide seed coating	Intercept	-36.53			
	Day	-1.61	1, 16	0.92	0.35
	Day × day	0.13	1, 14	10.78	0.0055
***A. mellifera* colony strength**					
All fields	Intercept	9834.46			
	Initial colony strength	-0.19	1, 64	1.67	0.20
	Treatment	-41.51	1, 7	0.01	0.94
Excluding the two field pairs with other spring sown oilseed rape field within 1 km	Intercept	9609.95			
	Initial colony strength	-0.18	1, 45	1.33	0.26
	Treatment	199.73	1, 5	0.11	0.76
Excluding the field pair where Biscaya was used at the control field	Intercept	9715.31			
	Initial colony strength	-0.16	1, 57	0.82	0.37
	Treatment	90.68	1, 6	0.02	0.88

Extended Data Table 7 | Number of individuals of wild bee species or groups at control (n = 8) and insecticide-treated (n = 8) oilseed rape fields

Group	Bee species	Control	Insecticide seed coating
solitary bee	*Andrena* sp.	15	25
solitary bee	*Colletes* sp.	5	2
solitary bee	*Hylaeus* sp.	1	0
solitary bee	*Lasioglossum/Halictus* sp.	10	3
solitary bee	*Macropis europaea*	1	0
solitary bee	*Nomada* sp.	1	3
solitary bee	*Sphecodes* sp.	4	1
solitary bee	unidentified solitary bee (not including *Osmia bicornis*)	10	0
bumble bee	*Bombus hortorum*	3	0
bumble bee	*Bombus hypnorum*	10	5
bumble bee	*Bombus lapidarius*	275	43
bumble bee	*Bombus pascuorum*	18	6
bumble bee	*Bombus pratorum*	3	6
bumble bee	*Bombus ruderarius*	2	2
bumble bee	*Bombus soroeensis*	1	0
bumble bee	*Bombus subterraneus*	1	0
bumble bee	*Bombus sylvarum*	2	0
bumble bee	*Bombus terrestris/lucorum/magnus/cryptarum*	712	403
bumble bee	unidentified bumble bee	190	159

Extended Data Table 8 | Residues of neonicotinoids (n) and a pyrethroid (p) in bee-collected pollen and nectar from control fields and fields sown with insecticide treated seeds

	Control (n = 8 fields*)		Insecticide seed coating (n = 8 fields)			
	Detected in n samples	Highest concentration	Detected in n samples	Highest concentration	LOD†	LOQ†
Honey bee pollen (ng/g)						
Acetamiprid (n)	1	0.34	0		0.080	0.24
Clothianidin (n)	0		8	23	0.50	1.5
Imidacloprid (n)	1	0.23‡	0		0.30	0.90
Thiacloprid (n)	3	1.4§	4	0.29	0.070	0.21
Thiamethoxam (n)	0		0		0.10	0.30
Beta-cyfluthrin (p)			0		1.0	
Honey bee nectar (ng/ml)						
Acetamiprid (n)	0		0		0.033	0.10
Clothianidin (n)	2	0.61	8	16	0.17	0.50
Imidacloprid (n)	3	0.35	0		0.17	0.50
Thiacloprid (n)	2	0.35§	2	0.044	0.033	0.10
Thiamethoxam (n)	1	0.19	0		0.17	0.50

* n = 6 for pollen collected by honeybees at control fields, because no such bees with pollen could be found at two fields.
† LOD, limit of detection; LOQ, limit of quantification.
Pollen LOD and LOQ were estimated from spiking experiments of the average sample weight of 0.056 g.
Nectar LOD and LOQ were estimated for the 0.016 ml sample volume.
‡ Sample weight of 0.091 g explains reported value slightly below the estimated limit of detection, based on a 0.056 g sample weight
§ One oilseed rape field sprayed with Biscaya (12 June 2013), where thiacloprid is the active ingredient (Extended Data Table 3).